Environmental Health Perspectives
Vol. 85, pp. 71–80, 1990

Type II Pneumocytes in Mixed Cell Culture of Human Lung: A Light and Electron Microscopic Study

by Lynne Bingle,* Terence B. Bull,* Bernard Fox,* Abraham Guz,* Roy J. Richards,† and Teresa D. Tetley*

Alveolar Type II epithelial cells dedifferentiate rapidly *in vitro*. Studies with animal tissue suggest that cell-cell and extracellular matrix-cell interactions are important in the retention of Type II cell morphology *in vitro*. Thus, in this study with human tissue, alveolar Type II cells, alveolar macrophages, and spindle cells were prepared from the same sample of lung (obtained following lobectomy for cancer, $n = 3$), cocultured on glass cover slips or tissue culture plastic, and studied by light microscopy with scanning (SEM) and transmission (TEM) electron microscopy for 8 days. The primary cell isolates contained approximately 45% Type II cells; the remainder were macrophages or unidentifiable cells. Clusters, made up of a single layer of cuboidal Type II cells around a central core of connective tissue (largely collagen and some elastic tissue), formed above a monolayer of spindle cells. The Type II cells were morphologically similar to those seen *in vivo*. The cells were still cuboidal at 8 days but had lost their lamellar bodies, which were released into the medium via the apical surface. The clusters increased in size with time (area, μm^2: day 1, 29(5–143) × 10^2; day 8, 63(10–311) × 10^2; mean(range); $p < 0.02$) without changing in number per culture, suggesting Type II cell proliferation. This may have been due to factors produced by the other cells and adherence to the extracellular matrix (ECM); (free collagen fibers, present in the original preparation, spindle cells, and/or Type II cells could be responsible for presence of ECM). We propose this as a useful model for the study of human Type II epithelial cells *in vitro*.

Introduction

The alveolar Type II pneumocyte is important in maintaining normal lung function, preventing alveolar collapse by the synthesis and secretion of surface active material (*1,2*), acting as a progenitor of alveolar Type I cells that are damaged during lung injury (*3,4*), controlling fluid and electrolyte transport (*5,6*), and producing components of the extracellular matrix (*7–9*). The Type II cell, therefore, plays an essential role in the homeostasis of the alveolar unit in health and disease.

Although it is possible to isolate and culture pure preparations of Type II cells from rodent (*10,11*) and human lung tissue (*12*), the study of isolated Type II cells is limited since these cells do not retain their cuboidal morphology when plated onto plastic. They lose lamellar body inclusions, their metabolic functions, and they dedifferentiate into intermediate or Type I cells (*10,12–14*). Neither do they divide in isolated cell culture (*13,14*). The normal morphology of Type II cells *in vitro* can be prolonged by plating onto exogenous extracellular matrices (ECM), such as laminin (*15*), collagen gels (*16*) or basement membrane derived from cultured cells (*15,17*), and other sources (*18,19*). Furthermore, macrophage- or fibroblast-derived factors are thought to influence the differentiated function of Type II cells *in vitro* (*17,20*).

Such studies illustrate the interdependence of different cell types and ECM in normal cell morphology and function. Thus, the development of an *in vitro* model to study Type II pneumocytes must consider the close proximity of the alveolar epithelial cell to mesenchymal and endothelial cells and the ECM produced by these cells *in situ*. Although some of these factors have recently been investigated using rat Type II cells (*7–9,15,17,19–21*), there are few similar studies using human tissue (*12*). In this preliminary study with human lung tissue, we have studied Type II pneumocytes—cocultured with spindle cells and macrophages obtained from the same parent tissue—by light and electron microscopy over a period of 8 days.

*Departments of Medicine and Histopathology, Charing Cross and Westminster Medical School, Fulham Palace Rd., London, W6 8RF, UK.
†Department of Biochemistry, University College, P. O. Box 78, Cardiff, CF1 1XL, UK.
Address reprint requests to T. D. Tetley, Department of Medicine, Charing Cross and Westminster Medical School, Fulham Palace Rd., London, W6 8RF, UK.

Materials and Methods

Tissue culture reagents were obtained from Gibco Ltd. (Paisley, Renfrewshire, UK). Osmium tetroxide was purchased from Johnson Matthey (Royston, Herts, UK). Propylene oxide was purchased from Agar Scientific (Stanstead, Essex, UK). Epoxy resin was obtained from Taab (Reading, Berks, UK). Cytokeratin PKKI monoclonal antibody and antimouse IgG horseradish peroxidase conjugate were purchased from Lab Systems (Uxbridge, Middx, UK), and Dako Ltd. (High Wycombe, Bucks, UK), respectively. Crude trypsin (type I, no. T-8003, lot 36F-8105) was purchased from Sigma Chemical Company (Poole, Dorset, UK). All other reagents were of AnalaR grade and obtained from Sigma Chemical Company (Poole, Dorset, UK) or British Drug Houses (Poole, Dorset, UK).

Human Lung Cell Culture

Tissue was obtained from lobectomy specimens removed for lung cancer ($n = 3$). Tissue was obtained from sites as far away from the tumor as possible. One or more airways were cannulated and 0.15 M NaCl instilled until the lung was fully inflated. The saline lavage was poured off and retained, and the process was repeated until the number of cells in the last lavage had fallen to less than 5% of those in the first lavage. The lung was reinflated by infusion with 0.5% trypsin, the cannula was fixed into position, and the lung suspended in 0.15 M NaCl at 37°C for 30 min. Any seepage of trypsin through the tissue during the incubation period was replaced via the cannula. Following this, the tissue was chopped into 1- to 2-mm pieces and suspended in fetal calf serum (FCS) and deoxyribonuclease (DNase; 250 mg/mL) to neutralize the enzyme and prevent cell aggregation. The suspension was filtered through sterile gauze and then 150 μm and 30 μm nylon mesh; this resulted in a cell suspension that was then layered onto a Percoll density gradient (1.04–1.09 g/mL) and centrifuged at 250*g* for 20 min at 4°C. Fraction 4 from the gradient was collected and diluted with a balanced salt solution containing 50 μg/mL DNase and centrifuged (*11*). The resulting cell pellet was resuspended in 10% FCS in low protein hybridoma media containing 100 U/mL penicillin, 100 μg/mL streptomycin, 0.05 mg/mL gentamycin, and 0.3 mg/mL glutamine.

A small portion of the cell suspension was cytospun for staining; the remaining cells were plated at 2×10^6 cells/well into 12-well (25 mm diameter) plastic culture dishes—some of which contained glass coverslips—for up to 8 days. The cells were maintained in an atmosphere containing 5% CO_2 and air, and the medium was changed every 24 hr. At regular intervals the cells were prepared for light and electron microscopy as described below. We were also able to plate cells from one of the tissue samples onto confluent, unpassaged, spindle-shaped cells obtained from a previous subject. These were stained for light microscopy at 24 and 48 hr.

Light Microscopy

Cells were identified at the light microscopic level by staining for alkaline phosphatase (AP), cytokeratin, and by the nitroblue tetrazolium (NBT) method.

Alkaline Phosphatase

The modified method of Miller et al. (*22*) was used for identification of epithelial Type II cells. Cytospun or cultured cells were allowed to dry in air then stained with naphthol phosphate and fast red (10 mg naphthol AS phosphate dissolved in 40 μL dimethyl sulfoxide then diluted in 10 mL 0.125 M 2-amino-2-methyl-1-propanol buffer, pH 8.9, containing 10 mg fast red) for 15 min at room temperature. The stain was washed off with distilled water, and the cells were counterstained in 1% methylene green for 30 sec and mounted in aqueous media.

Nitroblue Tetrazolium

The method described by Devereux et al. (*23*) was used to identify Clara cells. Cytospun or cultured cells were fixed in 10% buffered formalin for 40 sec. The slides were rinsed in Hepes PBS and incubated in 0.1% NBT and 0.1% NADPH in PBS at 37°C for 10 min. The cells were washed in distilled water, counterstained with 1% methylene green for 2 min, and mounted in glycerol.

Cytokeratin

In order to identify epithelial cells, the cultures were stained for cytokeratin. Cultured cells were fixed for 5 min in methanol and washed in Tween saline (0.15% Tween, 0.15 M NaCl). The cells were incubated with the anticytokeratin antibody (diluted 1:100 with 1 mg/mL bovine serum albumin [BSA] in PBS), for 45 min at room temperature then washed with Tween saline. The preparation was incubated with antimouse IgG conjugated to horseradish peroxidase (diluted 1:100 with 1 mg/mL BSA in PBS) for 45 min at room temperature and then washed with Tween saline. Following a PBS wash, the cell preparation was incubated with the substrate (0.01% H_2O_2 and diaminobenzidene in PBS) for 5 to 10 min, until a brown color developed and the reaction terminated by washing in distilled water.

Transmission Electron Microscopy

Cells were scraped off coverslips and centrifuged at 250*g* for 20 min at 4°C. The cell pellet was resuspended in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, and was left overnight at 4°C. The fixative was removed after centrifugation and the pellet resuspended in cacodylate sucrose buffer. The tissue was postfixed in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer for 1 hr at 4°C. The tissue pellets were then stained with 2% aqueous uranyl acetate for 30 min at room temperature, followed by dehydration to absolute alcohol. Propylene oxide was

used as a link reagent before embedding the cells in Taab epoxy resin.

Semithin sections (1–2 μm) were cut using an ultramicrotome with a glass knife and stained with toluidine blue in borax for light microscopic examination. Ultrathin sections (80 nm) of selected areas were mounted on copper grids, electron contrasted with Reynolds lead citrate, and examined with a Philips 201 transmission electron microscope.

Scanning Electron Microscopy

The cultures that were on tissue culture plastic were placed in Karnovsky fixative overnight at 4°C, then they were washed in cacodylate-sucrose rinse and dehydrated to absolute ethanol via graded alcohols. The preparations were critically point-dried using liquid CO_2, mounted on stubs, and sputtercoated with gold. The samples were examined with a Cambridge S200 scanning electron microscope.

Image Analysis

The slides stained for alkaline phosphatase (AP) were photographed, and 2 in. × 2 in. transparencies were prepared. These were projected onto a translucent, sensitized screen of a computerized image analysis system. A digitized pen was used to trace around the perimeter of the alkaline phosphatase positive (AP+) clusters of cells, and the number of clusters/mm² of cover slip and cluster area were calculated by reference to a 2 in. × 2 in. transparency of a micrometer scale that had been photographed on the same microscope and at the same magnification as the stained cells.

Results

The tissue from the three subjects weighed 30, 78, and 156 g; an average of 2.5×10^6 cells were isolated per gram of tissue. A mean of 43% (range 33–48%) of these cells were AP+ (Fig. 1*a*), while 45% (range 40–49%) were NBT positive (NBT+). Approximately 50% of the cells on the cytospin preparation for either the AP or NBT stain were negative.

At 24 hr the AP+ cells were forming clusters that had a mean area of 29×10^2 μm² that increased in size to a mean area of 63×10^2 μm² by day 8 (Fig. 1*b,c,d*; Table 1). The mean cluster size was significantly larger on day 8 compared to days 1, 2, and 3 ($p < 0.01$; Table 1), but it was not different from day 5. The mean cluster size on day 5 was significantly larger than that on day 2 ($p < 0.02$; Table 1).

The clusters of cells also stained for cytokeratin. The clusters staining for cytokeratin and AP appeared to overlay negative staining cells, which were composed of particle-laden macrophages and spindle-shaped cells (Fig. 1*b–d*). The spindle cells increased in number, often reaching confluence. The mean number of clusters per mm² of culture remained relatively constant (Table 1).

Table 1. Alkaline phosphatase positive cells in mixed human lung cell cultures.[a]

Day	*n*	Area, μm², × 10²	Numbers/mm² culture dish	
			Clusters	Single cells
Coverslip				
1	4	29 (5–143)	9.5 (6–19)	7.0 (0–28)
2	9	28 (4–95)	7.6 (2–16)	4.3 (0–35)
3	8	36 (2–187)	8.6 (3–22)	2.6 (0–15)
5	8	39 (6–265)*	9.4 (2–21)	13.0 (0–35)
8	6	63 (10–311)†	6.9 (2–18)	9.8 (0–36)
Monolayer				
1	3	18 (2–37)	32.9 (25–48)	58.0 (19–97)
2	2	44 (11–104)‡	54.1 (32–76)	32.0 (25–38)

[a]Cluster area and number of clusters per unit area of culture vessel. Data expressed as mean with range in parentheses; the data were analyzed using the nonparametric Wilcoxon rank sum test for unpaired data; n = number of 2 in. × 2 in. slides examined.

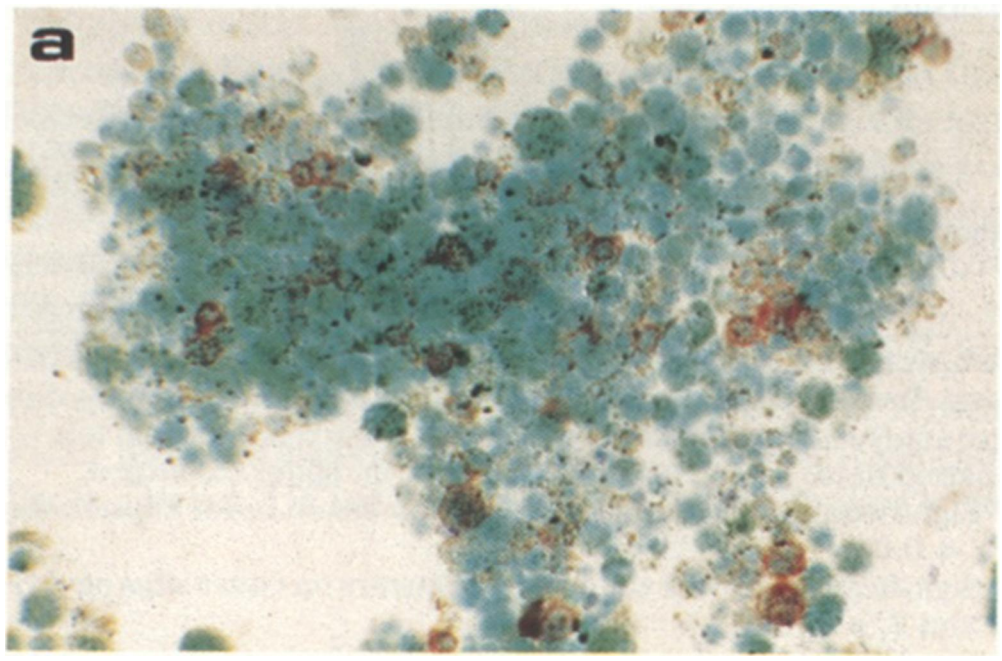
* Significantly greater than size of clusters on cover slips on day 2, $p < 0.02$;

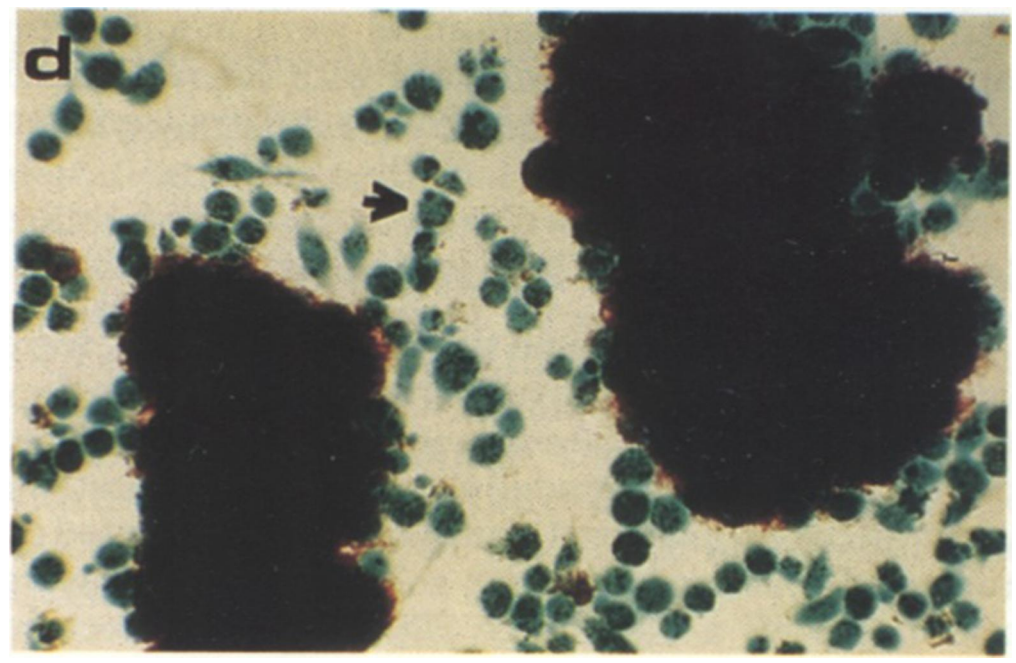
† Significantly greater than size of clusters on cover slips on days 1,2 and 3, $p < 0.01$;

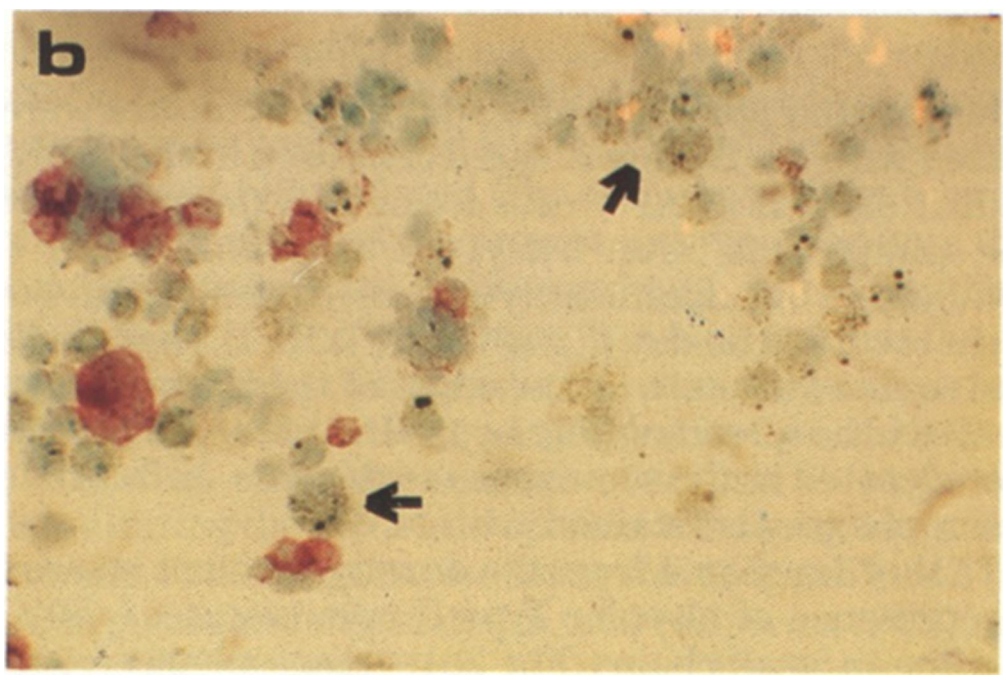
‡ Significantly greater than size of clusters on monolayers on day 1, $p < 0.001$.

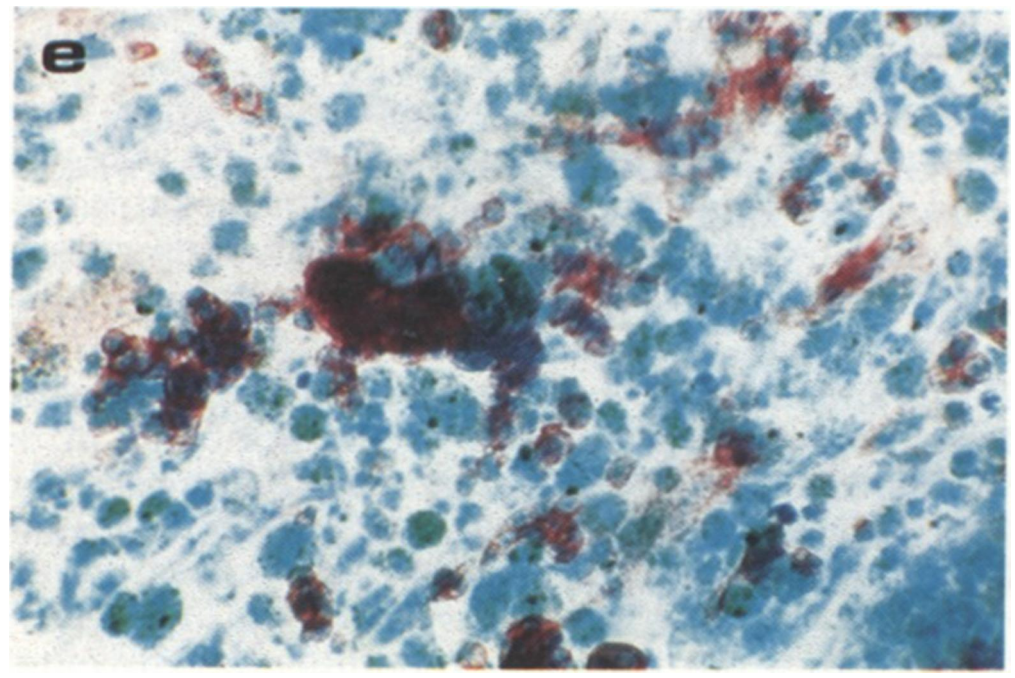
When confluent human lung spindle cell cultures were plated with the freshly isolated cells used in this study, similar clusters of AP+ cells formed on the surface of the spindle cells. After 2 days the areas of these AP+ cell clusters had significantly increased compared to the areas recorded on day 1 ($p < 0.001$; Table 1); single cells and some of the cells on the edges of these clusters appeared to be spreading (Fig. 1*e, f*). Most of the NBT+ cells were located in the same areas as the AP+ cells. Some macrophages also stained with NBT.

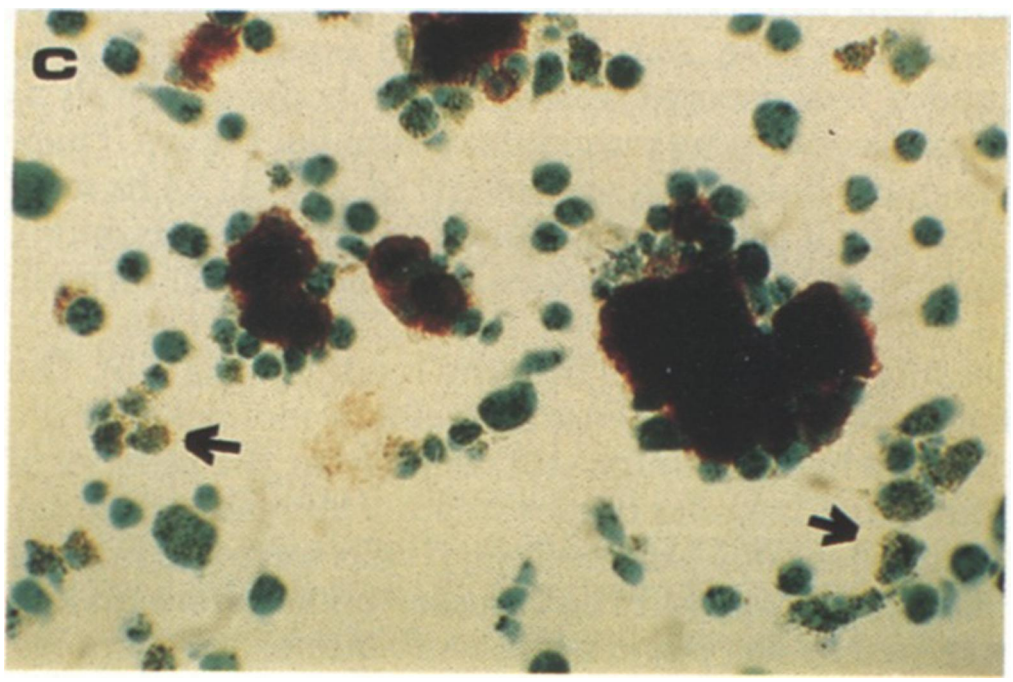
TEM of fraction 4 from the density gradient showed the presence of alveolar Type II pneumocytes (~ 50%) — often in small chains (Fig. 2*a*) — macrophages, some ciliated cells, and other cells that could not be identified. Clara cells were not found. Free collagen fibers were also present (Fig. 2*a*). The Type II pneumocytes contained a few recognizable lamellar bodies, as well as vacuoles containing remnants of lamellar bodies. There were many lipid-filled vacuoles (Fig. 2*a*). At 24 hr most of the Type II cells were cuboidal with numerous microvilli, and the lamellar bodies were large, well-formed, and full of myelin figures. By day 2, some cells were releasing myelin bodies into the medium (Fig. 2*b*). The lamellar bodies were dense and usually monocentric until day 3 when, although still very dense, the number of multicentric lamellar bodies steadily increased (Fig. 2*c*). The lamellar bodies of the Type II cells were still present at day 5 (Fig. 2*d*), but the lamellar bodies were greatly decreased by day 8 and there were numerous vacuoles, some of which contained remnants of myelin figures (Fig. 2*e*). Lipid vacuoles similar to those observed in the cells prior to culture were present at day 8 (Figs. 2*a,e*). From day 2 onward, Type II pneumocytes formed rings around a core of connective tissue containing large amounts of collagen and some elastic tissue (Figs. 2*d*, *e*, and *f*) and sometimes unidentifiable, possibly spindle cells. The cells in these structures formed tight junctions











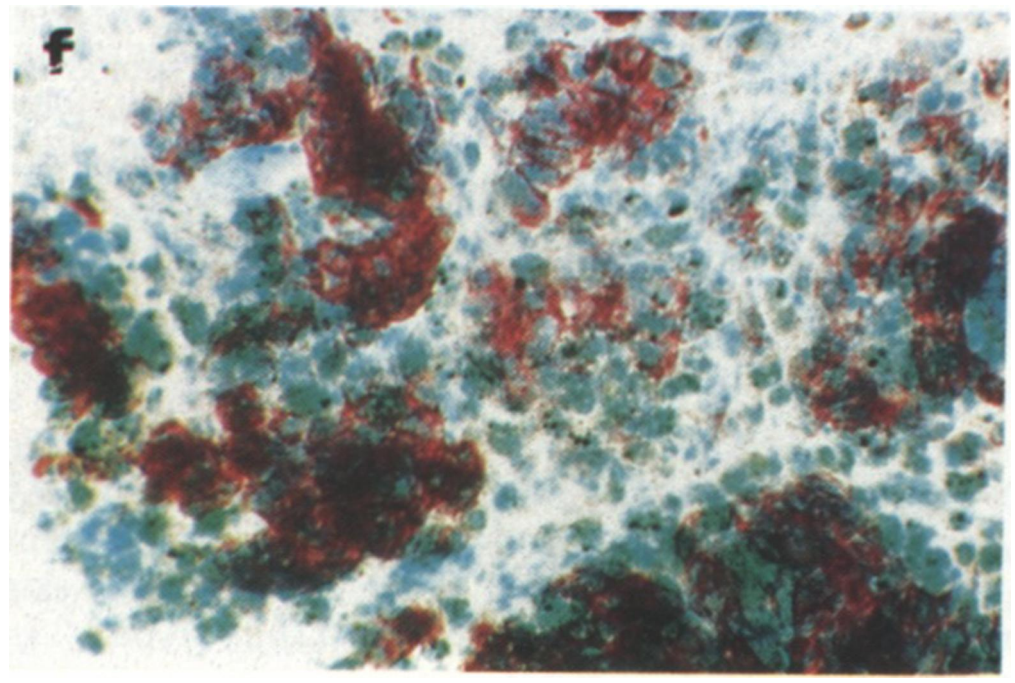


FIGURE 1. Alkaline phosphatase positive (AP+) cells (dark pink-red to black clusters) in mixed cells isolated from human lung tissue. (*A*) Preparation prior to culture. (*B*) 1 day, (*C*) 3 days, and (*D*) 5 days after plating onto glass; AP + clusters increase in size with time; the clusters are situated above negatively staining spindle cells and particle-laden macrophages (arrows). (*E*) 1 day, and (*F*) 2 days after plating onto monolayers; the clusters have increased in size, partly due to spreading cells at the edges; × 60.

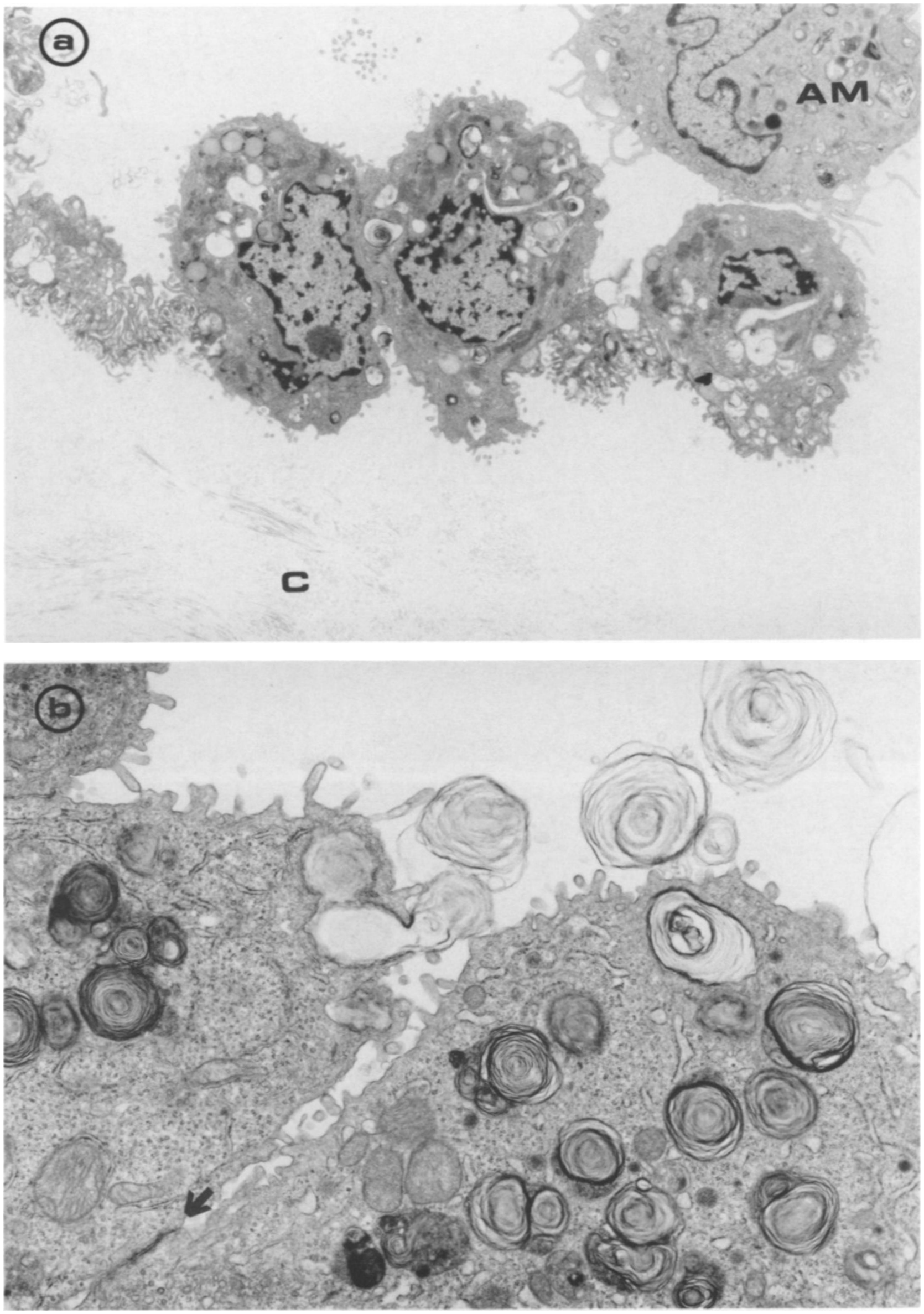


FIGURE 2. Transmission electron microscopy of (*a*) preparation prior to plating showing a string of three Type II cells containing lamellar bodies, some collagen fibers (C), and an alveolar macrophage (AM); × 5370. (*b*) Two days after plating; lamellar bodies free on the apical surface of the Type II cells. A tight junction is present between the cells (arrow); × 13,200. (*c*) At 3 days showing multicentric lamellar bodies (large arrows) among monocentric lamellar bodies, a tight junction (small arrow), and an alveolar macrophage (AM); × 7290. (*d*) At 5 days: a ring, mostly of Type II cells around a central core of connective tissue (CT) and two unidentified cells (UC). Lamellar bodies (arrows) are only present in some cells. The alveolar macrophage (AM) is vacuolated; × 2250. (*e*) By 8 days many of the cuboidal cells around the connective tissue (CT) core contain vacuoles, some of which contain remnants of myelin bodies; × 5050. (*f*) The core of connective tissue contains collagen (C), elastic tissue (E) and basement membrane (BM); × 14,760. (*g*) Collagen fibers present in close proximity to the cell membrane; the cells contain microtubules (arrows); × 35,250. *Continued on next page.*

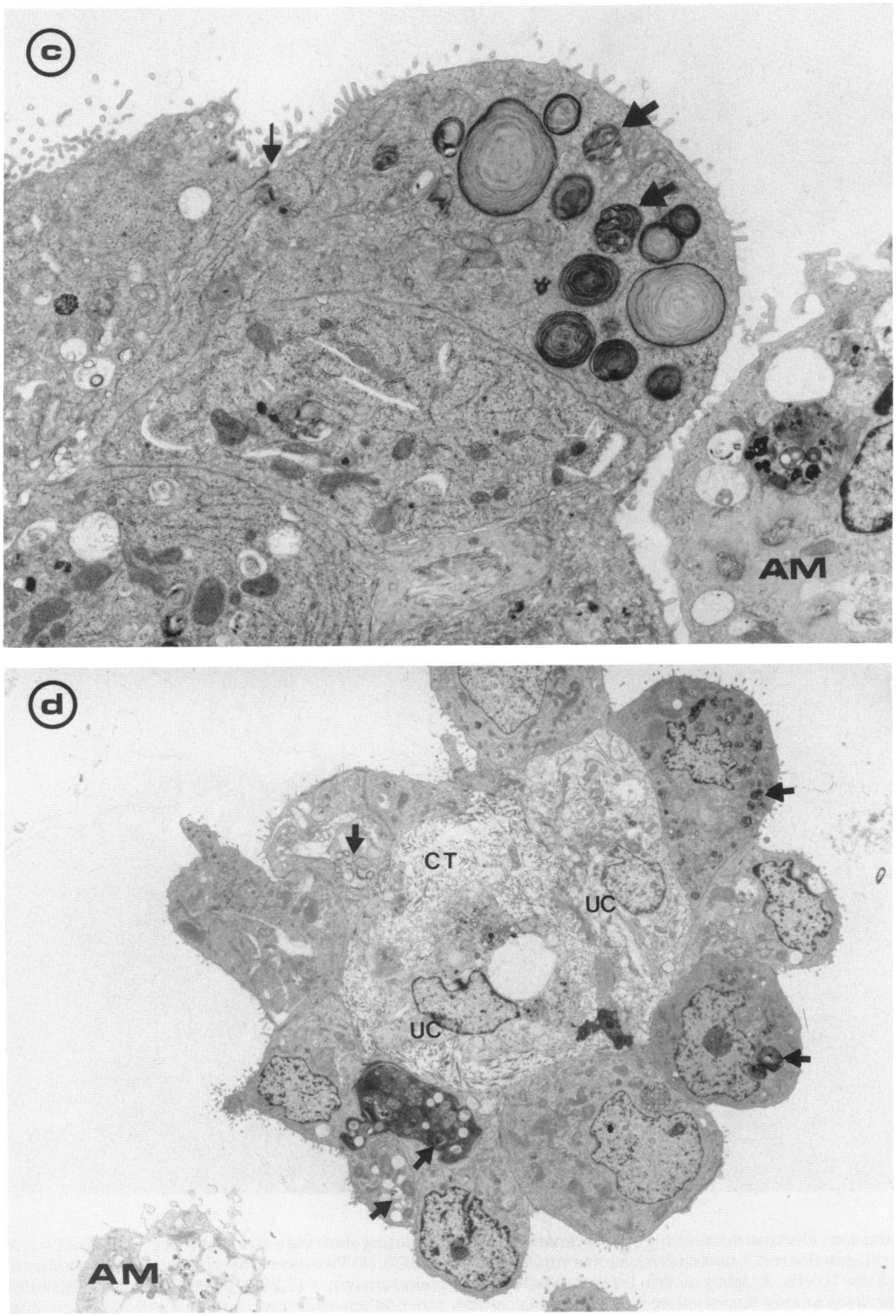


FIGURE 2. *Continued.*

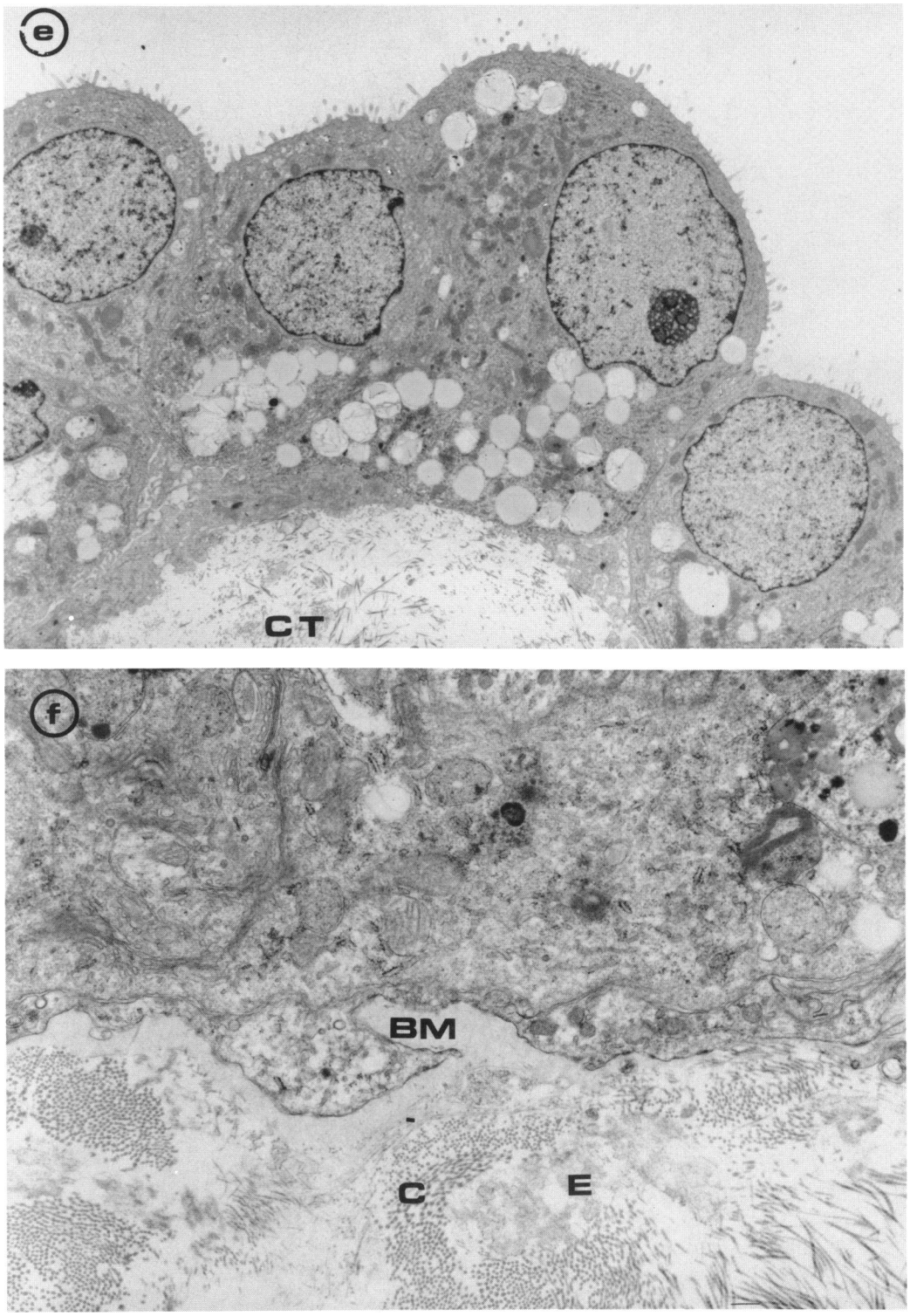


FIGURE 2. *Continued.*

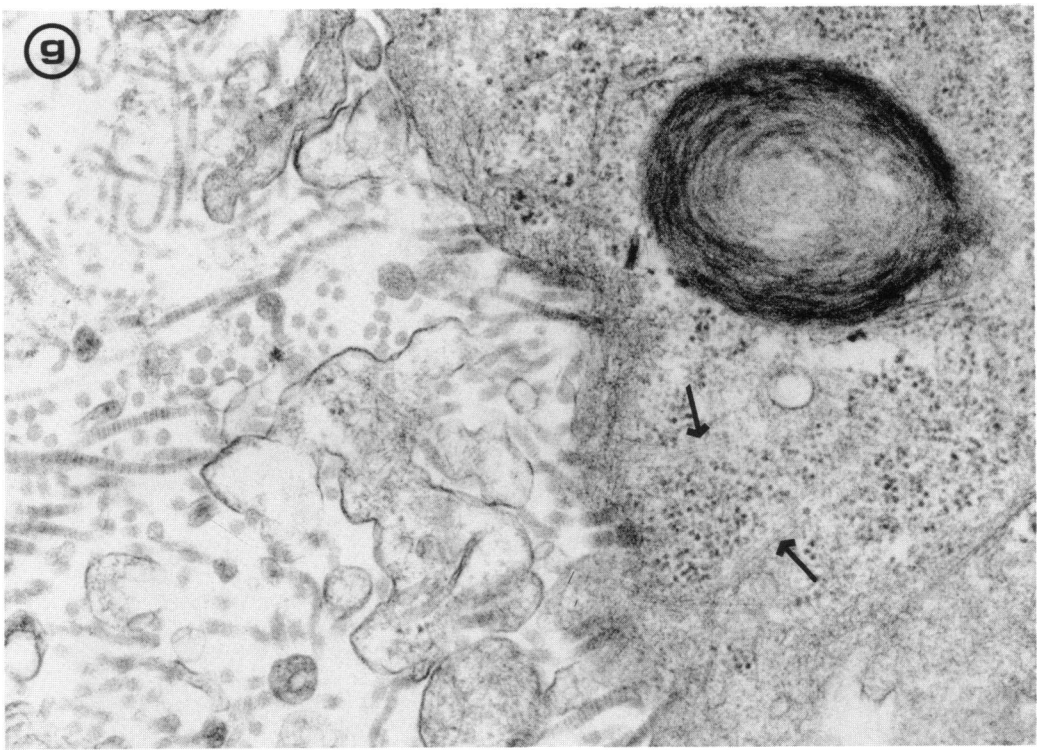


FIGURE 2. *Continued.*

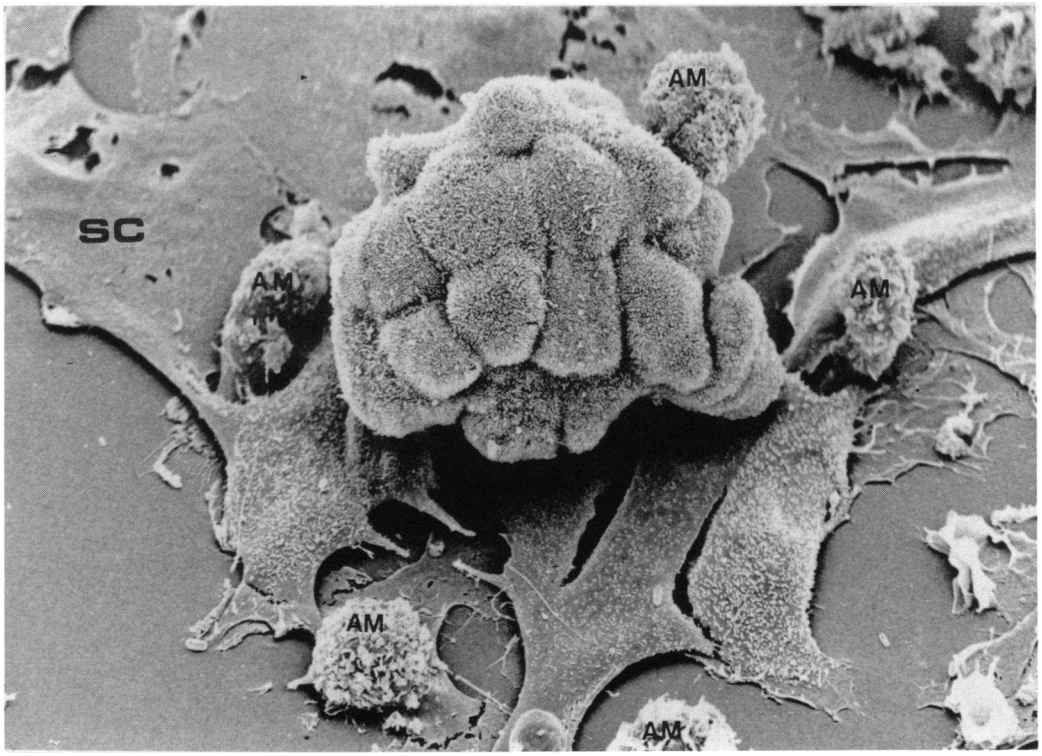


FIGURE 3. Scanning electron microscopy at day 3 showing cluster of Type II cells, covered in microvilli, above the spindle cell (SC) monolayer. Some alveolar macrophages (AM) are also present; ×1500.

(Figs. 2*b,c*). In places there was basement membrane that separated the cell from the underlying connective tissue (Fig. 2*f*). In some Type II cells, microtubules were seen, and the collagen fibers were intimately associated with the surface membrane of the Type II pneumocyte (Fig. 2*g*). Throughout the 8 days of culture macrophages were present, but these deteriorated in structure so that by day 5 they were mainly degenerate vacuolated cells (Fig. 2*d*). Within the clusters of Type II cells there were cells without myelin bodies, that had microvilli, and were cuboidal in shape (Figs. 2*d,e*). Only one Clara cell was positively identified by electron microscopy in this study.

Scanning electron microscopy (SEM) showed clusters of rounded cells covered with microvilli characteristic of Type II pneumocytes. These were situated above flattened monolayers of spindle shaped cells that were spreading on the culture vessel. Macrophages were also observed above the spindle cells in the proximity of the clusters (Fig. 3).

Discussion

In this study of cocultures of human alveolar Type II pneumocytes, alveolar macrophages, and spindle cells isolated from the same parent tissue, we observed the formation of clusters of Type II cells around a central core of connective tissue and above monolayers of spindle cells. Initially the Type II cells contained lamellar bodies, the contents of which were released via the apical surface into the culture medium. Most of these cells retained their cuboidal morphology during the 8 days of the study, although the number of lamellar body inclusions decreased during this time. As the clusters increased in size without a decrease in cluster number, it is possible that the Type II cells were undergoing cell division *in vitro*. However, no mitoses were seen, and no further studies have been undertaken to confirm or refute this hypothesis.

The appearance by light microscopy of clusters of rounded AP+ cells above negatively stained spindle-shaped cells suggested that the Type II cells had preferentially migrated to these regions and avoided glass and plastic. SEM, which provided a clear three-dimensional picture, confirmed the relationship of the clusters to the spindle cells. TEM confirmed that the cellular component of the clusters largely consisted of cuboidal Type II pneumocytes. Type II pneumocytes have been shown to retain their cuboidal morphology when cultured on various ECM substrates, in contrast to the loss of morphology and spreading seen on tissue culture plastic. In previous studies the substrates used were pure single components of ECM (*9,16*) or complete ECM from cells of known origin (*15–19*). However, in this study the exact source and composition of the extracellular matrix is unclear. The ECM may originally have been the fragments of connective tissue in the primary cell preparation. Alternatively, the spindle type cells, which adhere within 3 or 4 hr of plating, may have laid down sufficient extracellular matrix to later attract and encourage Type II cell aggregation. Spindle cells present in the core of the clusters as unidentifiable cells (Fig. 2*d*) may have continued to produce ECM. It is also likely that the Type II cells themselves synthesized and secreted connective tissue components. Collagen fibers were intimately associated with the surface of the Type II cells that contained abundant microtubules indicating the synthesis and secretion of macromolecules (Fig. 2*g*). Furthermore, it appeared that the central core of connective tissue within the clusters was getting larger with time; basement membrane formation, found in the later cultures, was not present at an earlier stage (Fig. 2*e*). Synthesis of basement membrane (*16,19*), as well as Type IV procollagen and fibronectin has been demonstrated in cultured rat Type II pneumocytes (*7–9*). All these molecules are essential components of the alveolar matrix.

There are at least three possible reasons for an increase in the size of clusters (Table 1) apart from the increase in the central connective tissue core: *a*) aggregation of Type II cells; *b*) proliferation of Type II cells; and *c*) flattening, without dividing, of Type II cells over the surface of the core. There was no evidence that aggregation (i.e., decreased cluster number with an increase in cluster size) was occurring (Table 1), nor that the Type II cells were becoming flattened (Fig. 2). There is circumstantial evidence that cell division may account for an increase in the size of clusters. That is, over 8 days there was a doubling in the size of the clusters that were usually covered only by a single continuous layer of largely cuboidal cells. The increase in cluster size was more marked when cells were plated onto monolayers, with a 2-fold increase in size within 24 hr. Although some of the latter increase in cluster size may reflect spreading of some of the cells on the edge of the clusters, most of the cells remained cuboidal. In neither situation was there a decrease in the number of clusters per unit area of culture, supporting the suggestion that the cells were dividing.

The finding that isolated Type II pneumocytes retain their cuboidal morphology longer when cultured on laminin or more complex ECM of known origin suggests that such compounds may be important in Type II cell proliferation, since cell morphology is thought to be directly related to cell function. In addition, there is evidence that the differentiated function and proliferation of Type II cells is stimulated by factors from macrophages, fibroblasts, and possibly other cell types (*17,20,24*). In the present study, a combination of coculture of human Type II pneumocytes with alveolar macrophages and spindle cells obtained from the same tissue sample, together with adherence of the Type II cells onto a connective tissue substratum, may have provided a situation in favor of Type II cell proliferation *in vitro*.

A recent study of rat Type II cells suggests that a pliable substrate assists in the retention of normal Type II cell morphology (*17*). In the present model, the core of

connective tissue may be relatively pliable, being free of the culture vessel and possibly manipulated by the Type II cells that have been shown to degrade ECM *in vitro* (*9*). Thus, the Type II cells themselves remodel the connective tissue core that, in turn, promotes their normal morphology. Nevertheless, it is important to remember that a number of studies have shown a retention of Type II cell morphology on nonpliable extracellular matrices, implying that the nature of the substrate is a dominating factor in the expression of cell phenotype.

The NBT stain has been used to differentiate Clara cells from Type II cells isolated from rabbits (*23*). This present study, using the same modified technique of Devereux et al. (*23*), shows that human Type II cells and macrophages stain with this dye. NBT+ macrophages may reflect the reduction of NBT by the products of high levels of diaphorases and superoxides produced by activated macrophages from smokers' lungs (*25*). It is also possible that products of the reaction of high levels of alkaline phosphatase with endogenous or exogenous substrates would reduce NBT to give a positive result (*26*). This would explain the present observation of NBT+ Type II pneumocytes and macrophages.

Summary

An *in vitro* model of human Type II pneumocytes cultured with spindle cells and macrophages from the same sample of human lung has been established. Clusters of cuboidal Type II cells are formed around a core of connective tissue on the surface of spindle cells. The Type II cells remain cuboidal for 8 days in culture, although the number of lamellar body inclusions decreases. The clusters increase in size; we suggest that this might be due to cell division, possibly under the influence of factors released by the other cells. This model needs to be studied further to establish whether active metabolism is occurring in these cells and whether they are undergoing division.

We would like to thank the Medical Research Council for funding L. Bingle and A. R. Makey MS, FRCS, for providing the lung tissue specimens.

REFERENCES

1. Pattle, R. E. Properties, function and origin of the alveolar lining layer. Nature 175: 1125–1126 (1955).
2. King, R. J. The surfactant system of the lung. Fed. Proc. 33: 2238–2247 (1974).
3. Adamson, I. Y. R., and Bowden, D. H. The Type II cell as a progenitor of alveolar epithelial regeneration. Lab. Invest. 30: 35–42 (1974).
4. Evans, M. J., Cabral, L. J., Stephens, R. J., and Freeman, G. Transformation of alveolar Type II cells to Type I cells following exposure to NO_2. Exp. Mol. Path. 22: 142–150 (1975).
5. Mason, R. J., Williams, M. C., and Widdicome, J. H. Fluid and electrolyte transport across monolayers of alveolar Type II cells *in vitro*. Am. Rev. Respir. Dis. 127: S24–S28 (1983).
6. Castronova, V., Jones, G. S., Wright, J. R., Colby, H. D., Bowman, L., and Miles, P. R. Transport properties of isolated Type II alveolar epithelial cells. Am. Rev. Respir. Dis. 127: S28–S32 (1983).
7. Crouch, E. C., Moxley, M. A., and Longmore, W. Synthesis of collagenous proteins by pulmonary Type II epithelial cells. Am. Rev. Respir. Dis. 135: 1118–1123 (1987).
8. Sage, H., Farin, F. M., Striker, G. E., and Fisher, A. B. Granular pneumocytes in primary culture secrete several major components of the extracellular matrix. Biochemistry 22: 2148–2155 (1983).
9. Rannels, S. R., Fisher, C. S., Heuser, L. J., and Rannels, D. E. Culture of Type II pneumocytes on a Type II cell-derived fibronectin-rich matrix. Am. J. Physiol. 253: C759–C765 (1987).
10. Kikkawa, Y., and Yoneda, K. The Type II epithelial cell of the lung. I. Method of isolation. Lab. Invest. 30: 76–84 (1974).
11. Richards, R. J., Davies, N., Atkins, J., and Oreffo, V. I. C. Isolation, biochemical characterization and culture of lung Type II cells of the rat. Lung 165: 143–158 (1987).
12. Robinson, P. C., Voelker, D. R., and Mason, R. J. Isolation and culture of human epithelial Type II cells. Am. Rev. Respir. Dis. 130: 1156–1160 (1984)
13. Mason, R. J., Dobbs, L. G., Greenleaf, R. D., and Williams, M. C. Alveolar Type II cells. Fed. Proc. 36: 2697–2702 (1977).
14. Diglio, C. A., and Kikkawa, Y. The Type II epithelial cells of the lung. IV. Adaptation and behavior of isolated Type II cells in culture. Lab. Invest. 37: 622–631 (1977).
15. Rannels, S. R., Yarnell, J. A., Fisher, C. S., Fabisiak, J. P., and Rannels, D. E. Role of laminin in maintenance of Type II pneumocyte morphology and function. Am. J. Physiol. 253: C835–C845 (1987).
16. Geppert, E. F., Williams, M. C., and Mason, R. J. Primary culture of rat alveolar Type II cells on floating collagen membranes. Exp. Cell Res. 128: 363–374 (1980).
17. Shannon, J. M., Mason, R. J., and Jennings, S. D. Functional differentiation of alveolar type II epithelial cells *in vitro*: effects of cell shape, cell-matrix interactions and cell-cell interactions. Biochim. Biophys. Acta 931: 143–156 (1987).
18. Mason, R. J., Williams, M. C., Widdicome, J. H., Sanders, M. J., Misfeldt, D. S., and Berry, L. C. Transepithelial transport by pulmonary alveolar Type II cells in primary culture. Proc. Natl. Acad. Sci. USA 79: 6033–6037 (1982).
19. Lwebuga-Mukasa, J. S., Thulin, G., Madri, J. A., Barrett, C., and Warshaw, J. B. An acellular human amniotic membrane model for *in vitro* culture of Type II pneumocytes: the role of basement membrane in cell morphology and function. J. Cell. Physiol. 121: 215–225 (1984).
20. Mason, R. J. Pulmonary alveolar Type II cells and adult respiratory distress syndrome. West. J. Med. 143: 611–615 (1985).
21. Lwebuga-Mukasa, J. S., Ingbar, D. H., and Madri, J. A. Repopulation of a human alveolar matrix by adult rat Type II pneumocytes *in vitro*. Exp. Cell Res. 162: 423–435 (1986).
22. Miller, B. E., Chapin, R. E., Pinkerton, K. E., Gilmore, L. B., Maronpot, R. R., and Hook, G. E. R. Quantitation of silica-induced Type II cell hyperplasia by using alkaline phosphatase histochemistry in glycol methacrylate embedded lung. Exp. Lung Res. 12: 135–148 (1987).
23. Devereux, T. R., and Fouts, J. R. Isolation and identification of Clara cells from rabbit lung. In Vitro 16: 958–968 (1980).
24. Stoner, G. D. Explant culture of human peripheral lung. In: Methods in Cell Biology, Vol. 21A. Academic Press, New York, 1980, pp. 65–77.
25. Park, B. H., Fikrig, S. M., and Smithwick, E. M. Infection and nitroblue-tetrazolium reduction by neutrophils. Lancet: 532–534 (1968).
26. Lewis, F. A., Griffiths, S., Dunnicliff, R., Wells, M., Dudding, N., and Bird, C. C. Sensitive *in situ* hybridization technique using biotin-streptavidin-polyalkaline phosphatase complex. J. Clin. Pathol. 40: 163–166 (1987).